

Natural History Studies for the Preliminary Evaluation of *Larinus filiformis* (Coleoptera: Curculionidae) as a Prospective Biological Control Agent of Yellow Starthistle

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ABSTRACT We studied the life history, geographic distribution, behavior, and ecology of *Larinus filiformis* Petri (Coleoptera: Curculionidae) in its native range to determine whether it is worthy of further evaluation as a classical biological control agent of yellow starthistle, *Centaurea solstitialis* (Asteraceae: Cardueae). *Larinus filiformis* occurs in Armenia, Azerbaijan, Turkey, and Bulgaria and has been reared only from *C. solstitialis*. At field sites in central and eastern Turkey, adults were well synchronized with the plant, being active from mid-May to late July and ovipositing in capitula (flowerheads) of *C. solstitialis* from mid-June to mid-July. Larvae destroy all the seeds in a capitulum. The insect is univoltine in Turkey, and adults hibernate from mid-September to mid-May. In the spring, before adults begin ovipositing, they feed on the immature flower buds of *C. solstitialis*, causing them to die. The weevil destroyed 25–75% of capitula at natural field sites, depending on the sample date. Preliminary host specificity experiments on adult feeding indicate that the weevil seems to be restricted to a relatively small number of plants within the Cardueae. Approximately 57% of larvae or pupae collected late in the summer were parasitized by hymenopterans [*Bracon urinator*, *B. tshitscherini* (Braconidae) and *Exeristes roborator* (Ichneumonidae), *Aprostocetus* sp. (Eulophidae), and unidentified species of Eurytomidae and Ormyridae]. This weevil may be a better choice than the other capitula insects already established in the United States, particularly in colder parts of the plant's range.

KEY WORDS biological control, host plant, phenology, life history, Lixinae

Seven species of insects (including one accidental introduction; Balciunas and Villegas 2001) have been introduced to the United States for biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae: Cardueae) (Piper 2001, Pitcairn et al. 2004). However, the plant's population has not been sufficiently reduced, and there is interest to find additional prospective agents (Pitcairn et al. 2006, Smith 2004c). A weevil, *Ceratopion basicorne* (Illiger) (Coleoptera: Apionidae), that develops in the root crown of rosettes has been evaluated and proposed for introduction (Smith 2007). A stem-feeding flea beetle *Psylliodes chalconera* (Illiger) (Coleoptera: Chrysomelidae) (Cristofaro et al. 2004a), a lacebug *Tingis grisea* Germar (Heteroptera: Tingidae), and a mite *Aceria solstitialis* de Lillo (Acari: Eriophyidae) (de Lillo et al. 2003) are also being evaluated (Smith 2004c).

To optimize the efficiency of a classical biological control project, it is important to conduct a rapid preliminary evaluation of prospective agents to identify those worthy of more intensive evaluation (McClay and Balciunas 2005). Such a preliminary evaluation should assess both host plant specificity and potential efficacy. This step is critical to minimize the costly risks of either discarding a species that could be safe and effective (Smith et al. 2008) or spending many years of evaluation that lead to either its rejection or the release of an agent that is ineffective. Thus, preliminary evaluation is at least as critical as the other phases in the development of successful biological control (Harley and Forno 1992). For the *C. solstitialis* project, scientists have extended exploration further east, which has led to the discovery of more prospective agents (Cristofaro et al. 2002, 2004). Of these, we need to determine which are most likely to be safe and effective, as well as complement the agents already introduced. This involves obtaining basic information on the natural history of the agents, their host plant specificity, and potential efficacy.

Yellow starthistle is an important invasive alien weed in rangelands of the western United States (Shelley et al. 1999, DiTomaso 2005, DiTomaso et al. 2006). It is a winter annual adapted to a Mediterranean cli-

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mate (mild wet winter, dry summer). Seeds usually germinate in autumn, after the start of winter rains, or in early spring. Rosettes develop during winter and spring and can withstand burial under snow. Plants produce flower stems (bolt) in late spring and continue flowering during the summer until the plant dies from desiccation or frost.

Turkey and Greece are considered to be the geographic center of origin of *C. solstitialis* because of the high number of closely related taxa that occur there (Wagenitz 1975). The insects associated with *C. solstitialis* in Greece have been well studied (Sobhian and Zwölfer 1985, Groppe et al. 1990, Clement and Sobhian 1991), and five seedhead insects were introduced to the United States. Explorations for prospective agents of yellow starthistle were also conducted in Turkey (Pemberton and Hoover 1980, Rosenthal et al. 1994) and more recently in Turkey and southern Russia (Cristofaro et al. 2002). Recent exploration in eastern Turkey also showed the presence of *Larinus filiformis* Petri, 1907 (Coleoptera: Curculionidae) (Cristofaro et al. 2002). This insect had not been reported during previous explorations for prospective agents of yellow starthistle (Zwölfer 1965, Pemberton and Hoover 1980, Sobhian and Zwölfer 1985, Clement and Mimmocchi 1988, Rosenthal et al. 1994). The genus *Larinus* includes at least two effective biological control agents of Cardueae plants: *L. minutus* Gyllenhal on *Centaurea diffusa* Lam. (diffuse knapweed) and *Centaurea stoebe* L. (spotted knapweed) (Seastedt et al. 2003, Smith 2004b, Story et al. 2004), and *Larinus latus* (Herbst) on *Onopordum* spp. (Scotch thistle) (Briese 2000). *Larinus curtus* Hochhuth was introduced to control *C. solstitialis* in the United States but has failed to achieve dense populations (Pitcairn et al. 2004, Pitcairn et al. 2005), possibly because of egg predation, inferior competitive ability, or *Nosema* infection (Villegas et al. 2000). Because some other members of the genus *Larinus* were effective agents, we thought that *L. filiformis* warranted further study as a prospective agent of *C. solstitialis*.

There is little published information available about *L. filiformis*. Petri (1907) described the species based on specimens from the Aras valley (Armenia). Ter-Minassian (1967) reported collecting the insect in "Transcaucasus (Araks Valley)". Nothing else was reported in the literature at the time we began our studies (Cristofaro et al. 2002, 2006, Gültekin et al. 2006). The goal of this study was to obtain basic information on the life history and ecology of *L. filiformis*, including geographic distribution, host plants, infestation level, and natural enemies that could help determine its suitability as a biological control agent.

Materials and Methods

Examination of Museum Specimens

The senior author examined museum specimens by either visiting or borrowing material from the museums listed below or personal collections. All available specimens of *L. filiformis* were examined. The muse-

ums were the Atatürk University Entomology Museum, Erzurum, Turkey (EMET), the Hungarian Natural History Museum, Budapest (HNHM), Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZIN), Natural History Museum, London, United Kingdom (NHM), Royal Institute of Natural Sciences, Brussels, Belgium (RBINS), and R. Borovec Collection, Sloupno, Czech Republic (RBC).

Field Surveys

From 2003 to 2005, we conducted field surveys from early spring (May) through midsummer (July), when adult activity ceased. The goals were to collect live adults for biological experiments and to observe hibernation places, initiation of adult activity in the spring, adult feeding, mating, oviposition, larval feeding and development, host plants, oviposition preference, impact of adults and larvae on *C. solstitialis*, and seasonal occurrence of different developmental stages and to collect associated parasitoids and entomopathogens. We periodically collected capitula (flowerheads), some of which were dissected immediately, whereas others were held to rear out adult insects. Surveys were conducted in 16 provinces in central and eastern Turkey (Adana, Adıyaman, Ankara, Bayburt, Bingöl, Elazığ, Erzincan, Erzurum, Hatay, Iğdır, Kars, Kayseri, Kırıkkale, Nevşehir, Niğde, Sivas).

The principal study sites were located in Bingöl Province (from 35 km northeast of Bingöl to 15 km west of Bingöl) and in Iğdır Province (from 6 km east of Tuzluca to 7 km east of Iğdır). Both provinces are temperate regions. The Bingöl region is characterized by *Quercus* forest with open areas, including abandoned fields where *C. solstitialis* commonly occurs. Iğdır province contains the Aras river valley and Ağrı mountain lowland area (Iğdır Plain). The Aras valley is quite desertified and eroded and dominated mainly by semidesert vegetation. We selected investigation sites based on the abundance of yellow starthistle. When we found a site with many yellow starthistle plants, we searched them for signs of *L. filiformis* (e.g., feeding and oviposition damage, and presence of adults; Fig. 1a–c). At each location, we collected weevils by beating plants over an open umbrella or by sweeping with an insect net. Depending on the abundance of plants at the location, we swept or beat 20–100 plants. At three locations, we periodically collected capitula to dissect in the laboratory to monitor development of immature stages (see Table 3 for locations, dates, and numbers). We recorded the weevil species associated with *C. solstitialis* for each location (Gültekin et al., unpublished data), described the habitat, and recorded the GPS coordinates. During 1997–2006, while conducting a survey of *Larinus* biodiversity, the lead author recorded host plant associations in eastern Turkey by examining many plants in the tribe Cardueae (Asteraceae), especially from the genera *Acroptilon*, *Arctium*, *Carduus*, *Carlina*, *Carthamus*, *Centaurea*, *Cirsium*, *Cousinia*, *Echinops*, *Jurinae*, *Onopordum*, *Serratula*, and *Silybum*. Mature capitula were

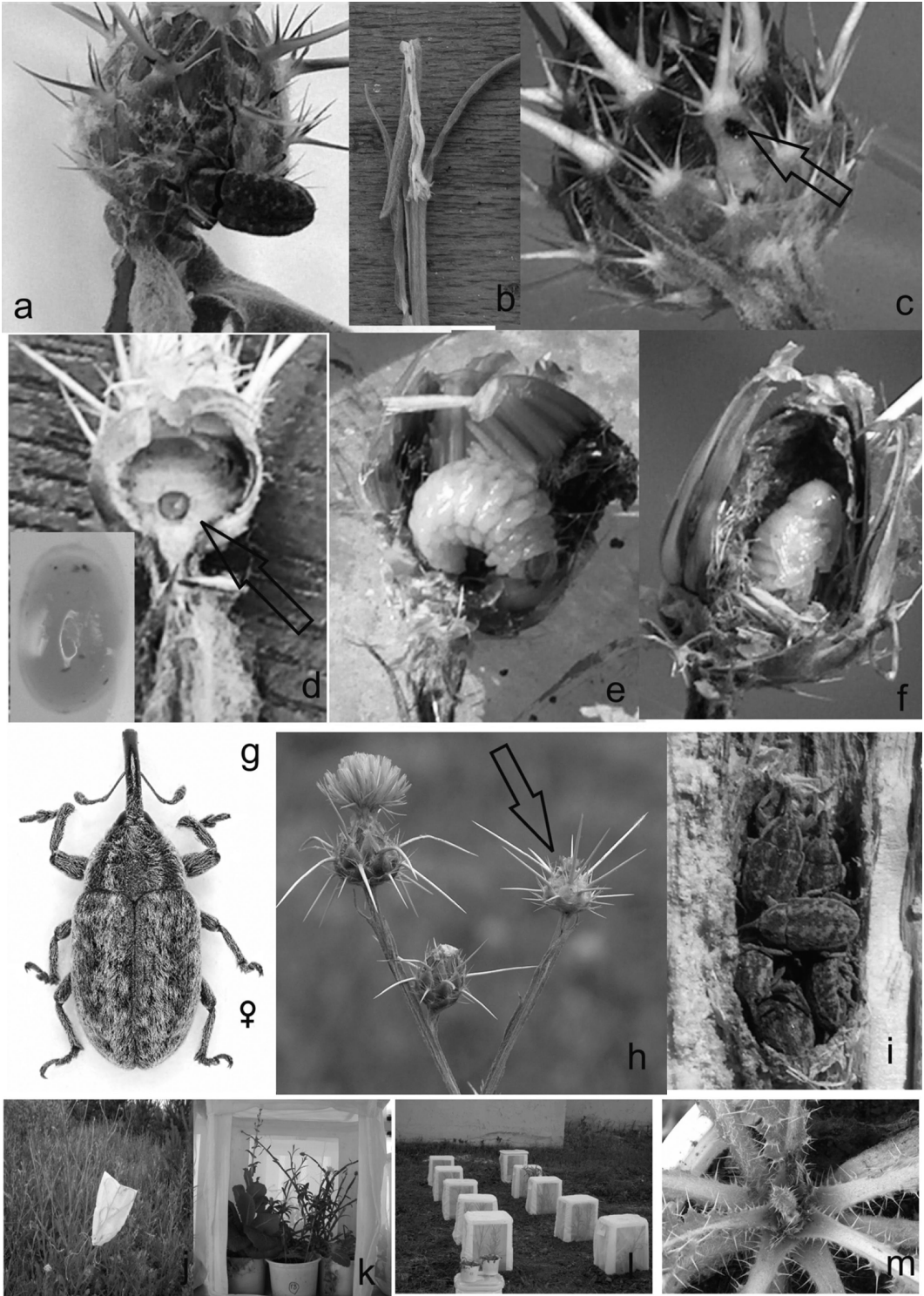


Fig. 1. Photographs of *L. filiformis*: (a) adult feeding on *C. solstitialis* capitulum, (b) adult feeding damage on young stem, (c) oviposition site, (d) egg, (e) mature larva that has consumed all seeds in a capitulum, (f) pupa, (g) adult (female), (h) infested capitulum compared with uninfested capitula, (i) aggregated hibernating new generation adults at late autumn, (j) sleeve bag on *C. solstitialis* in field experiment, (k) choice experiment in climate chamber, (l) field experiment cages, and (m) damage to *C. brevistylum*.

collected and held to rear adult insects from known host plants.

Behavior of Newly Emerged Adults

We randomly collected a total of 417 mature and dry capitula at two locations, "İğdir-Tuzluca" and "İğdir-western slope," in eastern Turkey 22 July 2003 to rear adults and possible parasitoids. The capitula were placed in transparent plastic bags (25 by 40 cm, 50 capitula per bag) that were ventilated with small pinholes and were held at room conditions (20–25°C and ≈50% RH). A few adults emerged by themselves, but they usually could not exit dry capitula under laboratory conditions, so we dissected capitula on five different dates between 22 July and 16 September. A total of 42 adult *L. filiformis* reared from field-collected capitula during 11–16 September were placed in a screen cage (28 by 28 by 30 cm). The wood floor of the cage was covered with sandy soil 5 cm deep. The cage contained a bouquet of live *C. solstitialis* stems, dry plant stems of *Crambe orientalis* L., and stones to imitate natural conditions. Adult behavior (feeding, mating, location, and aggregation) was observed for 1 wk after 16 September, and adults were put in a refrigerator to keep alive for future experiments.

Adult Feeding Behavior Experiments

We collected adult *L. filiformis* in the field in the spring as they were emerging from hibernation to use in experiments to observe feeding and oviposition behavior. Sex was determined by examination under a microscope. First and second visible ventrites of male are always depressed longitudinally in median; general appearance of complete venter is slightly concave; venter of female is convex and has no depressed area on first and second visible ventrites; rostrum of female distinctly longer than male. Male and female pairs were held in a transparent empty plastic tube to observe mating behavior and duration. Host specificity experiments, under both choice and no-choice conditions, were conducted in an electronically controlled climate chamber (25°C, 50% RH, 12:12-h light:dark photoperiod) at Atatürk University in Erzurum, Turkey. All test plants were grown from seed planted in flower pots (10 by 12 cm) on 8 March 2003 in the climate chamber (Erkam Teknik, Erzurum, Turkey; model: Evap/FEM; model number: RP-400514/54; size: 12.9 m²). Most of the plants had capitula in the young bud, preflowering, and flowering stages at the time of exposure to the adult insects, but some test plants were still in the rosette stage despite growing at the same conditions in the climate chamber (see Results and Discussion). Each capitulum was dissected under the microscope, and the percentage of tissue consumed was visually estimated using categories (0, 25, 50, 75, and 100%).

I. Adult Feeding on Host. One female *L. filiformis* (Fig. 1g) collected at Bingöl on 20 May 2003 was placed with one to three males in each of eight nylon mesh cages (35 by 50 by 40 cm) in a climate chamber

(25°C, 50% RH, 12:12-h light:dark photoperiod). Each cage contained two *C. solstitialis* bolted plants: one grown from seed collected at Aşkale, Turkey and the other from either California or Çat, Turkey. The experiment was conducted from 20 May to 16 June 2003. At the end of the experiment, the developmental stage of each capitulum was classified according to the following classes that correspond to the phenological categories of Maddox (1981): bud, Bu-1 or Bu-2; pre-mature, Bu-3; mature, Bu-4; preflowering, F-1; flowering, F-2; postflowering, dry capitulum. Adult feeding damage and oviposition were recorded. Capitula were held in individual containers at room temperature to rear out adult insects. The mean number of capitula available per cage was 19.5 ± 8.7 (SD) (range, 5–34).

II. Choice Experiment—Single Female-Male Pair. The experiment was conducted at the same conditions as above, except that four to five test plants of different types were put in each nylon mesh cage (35 by 50 by 40 cm; Fig. 1k). Each test included one *C. solstitialis* plant from California seed and one from either Aşkale or Çat seed. Two to three nontarget test plants were included in each test from among the following: *Centaurea americana* Nutt. (American basketflower), *Ce. calcitrapa* L. (purple starthistle), *Ce. cyanus* L. (bachelor's button), *Ce. rothrockii* Greenm. (Rothrock's basketflower), *Carduus pycnocephalus* L. (Italian thistle), *Carthamus tinctorius* L. (safflower; linoleic variety), *Cirsium brevistylum* Cronq., *Ci. occidentale* (Nutt.) Jepson variety *candidissimum* (Greene) J.F. Macbr., and *Lactuca sativa* L. (lettuce). One female and male *L. filiformis* collected at Bingöl on 19 or 26 May 2003 were placed in each of five cages. The experiment was conducted from 20 May to 26 June 2003.

III. Choice Experiment—Multiple Females. Eleven females and 14 males collected in the Aras Valley on 12 June 2003 were released in a climate chamber (1 by 1 by 1 m, 25°C, 50% RH, 12:12 h light:dark photoperiod; Fig. 1k) with 106 plants, representing 12 species of plants, all in the rosette stage. Test plants included the same species as in the preceding experiment with the addition of *Ce. diffusa*, *Ce. stoebe* L. (often misnamed as *Ce. maculosa* Lam.), and *Onopordum acanthium* L., and the exclusion of lettuce. The experiment ran from 13 to 27 June 2003.

Sleeve Cage Field Experiment

A field experiment to measure the duration of adult mating and oviposition activity was conducted in the Atatürk University Campus field, 4 km west of the Agricultural Department (Erzurum, Turkey). The field had a dense population of *C. solstitialis* and *Xeranthemum annuum* L. Eight sleeve cages were tied to branches of *C. solstitialis* (Fig. 1j). One male-female pair was placed in each sleeve cage on 28 May 2003, and they were moved to a fresh branch every 4–5 d.

Development Time

To determine egg development time, stems containing capitula with oviposition scars were removed

Table 1. Regions and dates of searches for *L. filiformis* on yellow starthistle in Turkey during 2003–2005

Province	Elevation (m)	Dates	No. observations ^a	No. adult specimens of <i>L. filiformis</i>
Adana	1,026	29 June 2005	1	0
Adıyaman	850	5 June 2004	1	2
Ankara	918–1,174	20–23 June 2005	4	4
Bayburt	1,720	18 June 2004	1	0
Bingöl	1,200–1,500	6, 19, 26 May, 8 June, 3 July 2003, 4 June 2004	9	60
Elazığ	950–1,200	4 June 2004, 31 May 2005, 3 July 2005, 3 June 2006	6	57
Erzincan	1,400	17 June 2003	1	1
Erzurum	1,650–1,900	4 May 2003, 6 May 2003	2	0
Hatay	20	6 June 2004	1	0
Iğdır	1,020–1,125	12 June 2003, 22 July 2003, 23 July 2003	4	17
Kars	1,450	4 May 2003	1	0
Kayseri	1,020–1,340	9, 23 June 2004	6	12
Kırıkkale	672–696	20 June 2005	2	3
Nevşehir	950–1,350	8–9 June 2004, 23 June 2004	8	40
Niğde	1,720	8 June 2004	1	0
Sivas	1,150–1,260	9 June 2004	3	36

^a An observation consists of one location on one date.

1 d after exposure to adult *L. filiformis* in field cages (Fig. 11). The capitula were on stems 10–15 cm long, which were placed in a plastic jar containing water and held at room temperature (20–25°C). Each day the phyllary (bract on the capitulum) covering the egg was pried open to determine time of larval eclosion. Larval development and seasonal period of activity were determined by dissection of capitula from survey field collections (described above). To determine pupal development time, eight mature larvae collected during late summer dissections were kept inside their capitula and placed in individual plastic jars held at room temperature. The insects were examined daily under a microscope by opening and closing some phyllaries.

Statistical Analysis

For proportion data, lower and upper 95% confidence intervals were calculated from the binomial distribution (Conover 1980). Differences in proportion data, such as sex ratio, proportion of capitula damaged in adult feeding experiments, and proportion of capitula of different developmental stages, were tested by χ^2 homogeneity tests.

Results and Discussion

Geographical Distribution

We conducted nine surveys in 2003, two extensive surveys in 2004, and one extensive survey in 2005 (Table 1). *L. filiformis* was previously collected in Armenia, Azerbaijan, Turkey, and Bulgaria (Petri 1907 Ter-Minassian 1967, Gültekin et al. 2006) (Fig. 2; Table 2). In Turkey, we collected it throughout a large geographic area comprising 10 provinces from the Armenian border (Iğdır prov.) to the western Mediterranean part of Turkey (Antalya prov.). However, the weevil may occur in a wider region beyond the locations we visited. We found it at 75% of the sites surveyed. It occurred at elevations from 672 to 1,900 m, and was most common (>80% occurrence) between 1,000 and

1,400 m (mean: 1147 ± 207 [SD] m; Fig. 3) and from 37° to 42° N latitude. In North America, *C. solstitialis* occurs from ≈ 32 –49° N latitude (Sheley et al. 1999), so this insect is expected to be adapted to the higher elevations and more northerly part of the plant’s distribution. The latitudinal distribution of the insect in Turkey, from 37 to 40° N latitude, suggests that it should be well adapted regarding seasonal synchrony by photoperiod over most, if not all, of the range of *C. solstitialis* in North America. This agent seems to prefer a higher, cooler habitat (where snow occurs) than the other established insects, which originated from Greece (Maddox et al. 1991, Pitcairn et al. 2003) and therefore would probably provide the greatest benefit at higher elevations and in the northern part of the plant’s distribution. Matching the climate of Sivas, where the largest number of specimens per sample were collected, using the computer program CLIMEX (v. 1.1, Sutherst et al. 1999), indicates that the mountains and foothills of eastern and northern California, eastern Oregon and Washington, and western Idaho are the most similar regions in the United States where yellow starthistle is invasive (Fig. 4). This geographic distribution suggests that the insect may be better adapted to cooler, higher elevations than the other insects previously introduced (Pitcairn et al. 2003). Also, *L. filiformis* is less likely to be effective in the warmer central valley and coastal hills of California, where yellow starthistle is most invasive.

Host Range

In the field surveys, we found *L. filiformis* adults and larvae only on *C. solstitialis*. Field observations and the dissection of >5,000 capitula from Cardueae plants during 10 yr indicate that *L. filiformis* larvae develop only on *C. solstitialis*. We did not find adults feeding on any other plant species, including: *Centaurea cal-citrapa*, *Ce. iberica* Trev ex Spreng., *Ce. virgata* Lam., *Ce. carduiformis* DC., *Ce. cyanus* and *Ce. polypodiifolia* Boissier, *Ce. kurdica* Reichardt, *Ce. behen* L., *Ce. ur-*

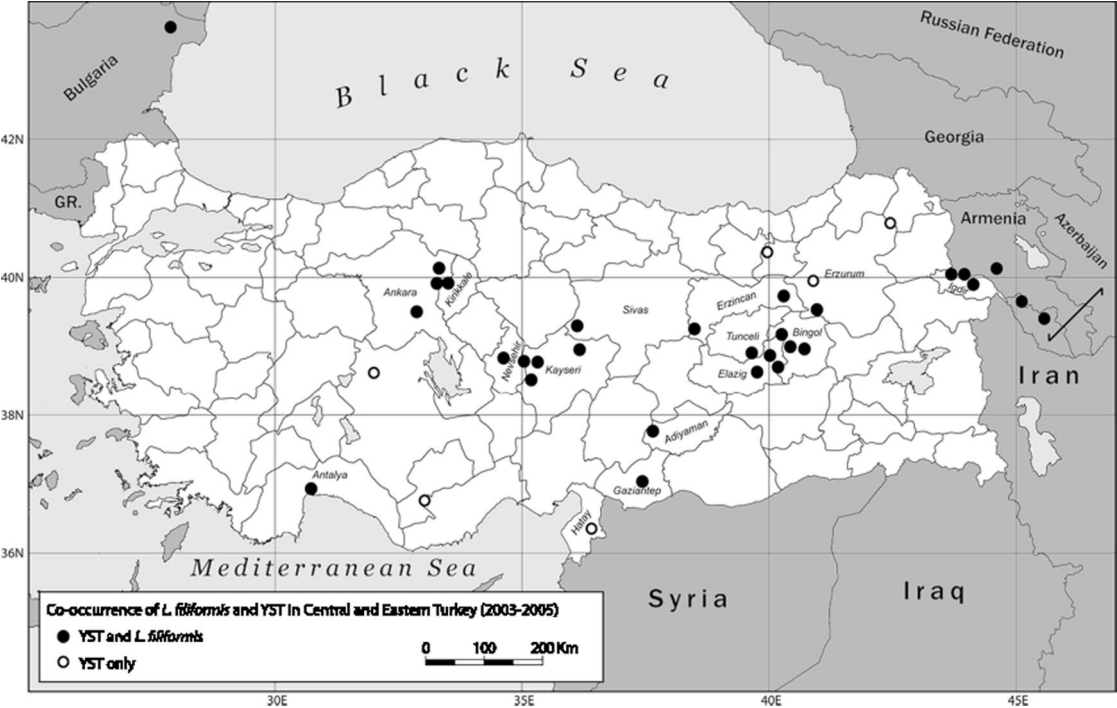


Fig. 2. Geographic distribution of *L. filiformis* based on our samples and other reported records. The insect occurs in Armenia, Azerbaijan, Turkey, and Bulgaria.

villei DC., *Carduus nutans* L., *Ca. pycnocephalus* L., *Picnomon acarna* L. (Cass.), *Carthamus lanatus* L., *Crupina crupinastrum* (Moris) Vis., *Echinops sphaerocephalus* L., *Onopordum bracteatum* Boiss. et Heldr., and *O. acanthium* L. *L. filiformis* is one of the most common insects on capitula of *C. solstitialis* in eastern Turkey, occurring at 83% of sites sampled during its season of occurrence (19 May to 23 July). In compar-

Table 2. Distribution and host plant record of *L. filiformis* Petri from museum specimens

Locality	Date	Altitude (m)	No. specimens and collector	Host plant	Museum or collection
Turkey					
Iğdır Prov., 10 km E of Tuzluca	17 June 1997	—	1: B. Korotyaev	—	EMET
Erzurum Prov., 9 km S of Çat	12 July 2001	1,900	1: L. Gültekin	YST	EMET
Erzincan Prov., Kemalîye, Sandıklı Vill.	20 June 1981	—	1: H. Özışık	—	EMET
Bingöl Prov., 35 km NW of Bingöl, Çobantaşı	2 June 2001	1,500	2: L. Gültekin	YST	EMET
Bingöl Prov., 12 km N of Bingöl	3 June 2001	1,200	1: L. Gültekin	YST	EMET
Tunceli Prov., Kızılçık	6 June 1992	—	1: S. Kadlec	—	RBC
Gaziantep Prov.	10 June 1999	—	1: M. Cristofaro	—	EMET
Nevşehir Prov., 5–15 km N of Gülşehir	19 June 1999	—	1: B. Korotyaev, 2: B. Korotyaev	YST	EMET, NHM
Antalya Prov.	19 July 1996	—	1: S. Başak	—	EMET
İçel Prov., Silifke, Törörorszag	8 June 1977	—	1: A. Podlussany	—	RBINS
Azerbaijan					
Nakhichevan Republic, Shakhbuz between Makmudaba Vill.	21 May 1988	—	1: B. Korotyaev	—	EMET
Nakhichevan Republic, Ilychovsk Distr., 6 km NW of Arpaçay Dam	4 June 1988	—	2: G. Davidian	—	EMET
Armenia [Caucasus]					
Araxesthal	—	—	1: Leder-Reitter (Syntype)	—	ZIN
Araxesthal	—	—	1: Leder-Reitter (Syntype)	—	HNHM
Yerevan	3 July 1988	—	1: A. Zamotaylov	—	EMET
Bulgaria					
Lozenec	14 July 1978	—	1: Laibner	—	RBC

YST, *Centaurea solstitialis*; EMET, Atatürk University Entomology Museum, Erzurum, Turkey; HNHM, Hungarian Natural History Museum, Budapest; NHM, Natural History Museum, London, United Kingdom; RBINS, Royal Institute of Natural Sciences, Brussels, Belgium; RBC, R. Borovec Collection, Sloupno, Czech Republic; ZIN, Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

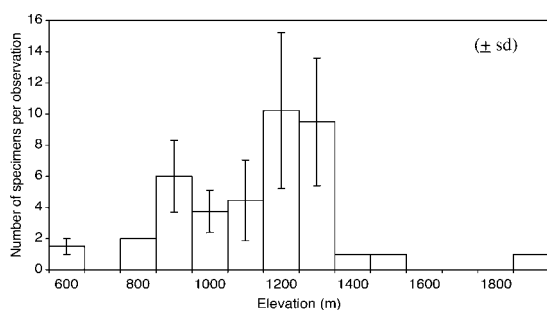


Fig. 3. Elevational distribution of *L. filiformis* based on our samples in Turkey. An observation consists of one location on one date. Error bar is \pm SD.

ison, the occurrence of other *C. solstitialis* capitulum insects was: *Eustenopus villosus* (Boheman) 33%, *Ban-gasternus orientalis* (Capiomont) 28%, and *Larinus curtus* 15% of sites.

Seasonal Activity and Biological Notes

The first adult activity observed in the spring was a male feeding on a rosette of *C. solstitialis* on 19 May 2003 (20 km northeast of Bingöl, 1,250 m elevation). On the same date at a nearby location, we found eight adults (four male, four female) 3–4 mm deep in soil under *C. solstitialis* rosettes (two or three individuals per plant). Weevils were active and started walking after they were caught. All *C. solstitialis* rosettes where the insects were found had round feeding holes (2–3 mm diameter) in the leaves, similar to what was observed in the laboratory experiments. It seemed that the adults emerged to feed when the temperature was $\approx 20^{\circ}\text{C}$ and returned to the soil when the temperature decreased. On the same date at a nearby location, an

individual that was still dormant was found under a stone. When these adults were put in the rearing experiment cages ($\approx 25^{\circ}\text{C}$, 50% RH, 12:12 h light-dark period), they started feeding on *C. solstitialis* leaves. Between 19 and 26 May, we collected 47 specimens (34 male, 13 female). The male:female ratio of 2.6 (Ho: 50% females; $\chi^2 = 9.4$; $\text{df} = 1$; $P < 0.005$) suggests that males became active earlier than females. Mating was not observed in the field on this date, but when we put specimens in a plastic bag to carry them to the laboratory, most started to mate. When pairs of males and females were placed together, they started mating within 3–25 min., and the copulation period ranged from 45 to 185 min. Females sometimes mated with more than one male, and males competed with each other, sometimes interrupting a copulating pair. Mating behavior continued during most of the adult life span, until the middle of July (17 July 2003, in field sleeve cage). The adults fed on young flower buds, the central growing tip of the rosette plant, and leaves of *C. solstitialis*. Adults often fed on the stem just below capitula, which caused the capitulum to die (Fig. 1b). Later in the season, they fed on the internal receptacle tissue and flower parts of immature and mature capitula using their long rostrum. Feeding holes were generally made in the lateral or bottom part of the capitulum (Fig. 1a,c). Overwintered adults were active in the field from 19 May to 3 August 2003 (Fig. 5). Adult females and males survived 61.0 ± 5.3 (SD) days (range, 48–75, $n = 21$) in the field sleeve cage experiment (starting from collection on 19 May).

Based on our observations in the field and cage studies, three conditions were necessary for oviposition to begin: temperature above 25°C , feeding for ≈ 3 wk, and availability of mature *C. solstitialis* capitula to feed on. We observed the first egg on 12 June 2003 in

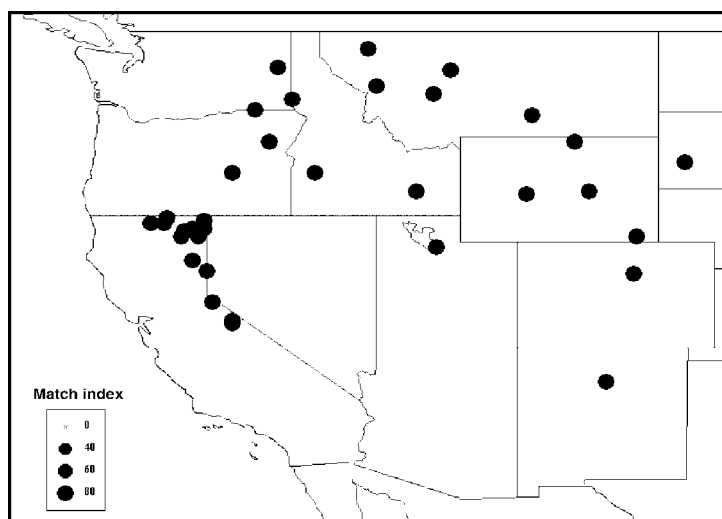


Fig. 4. Similarity of long-term climate of locations in the western United States compared with Sivas, Turkey, the site where the most specimens of *L. filiformis* were collected per survey observation, using Match Climates in the computer program CLIMEX (Sutherst et al. 1999). This geographic distribution suggests that the insect may be best adapted to cooler, higher elevations than the other insects previously introduced.

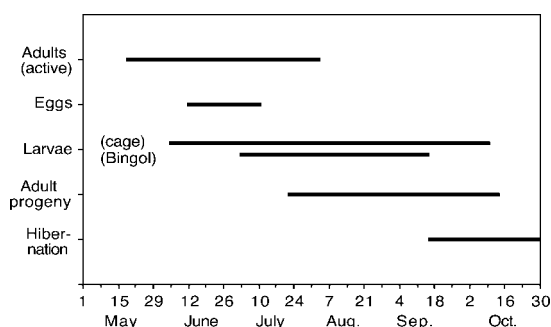


Fig. 5. Seasonal occurrence of *L. filiformis* developmental stages in eastern Turkey. (Cage refers to field-collected adult insects that were held with *C. solstitialis* plants in outdoor cages at Erzurum, Turkey).

Aras Valley (Fig. 1d). The female drilled an ovate hole with her rostrum in the lower part of capitulum, penetrating the receptacle tissue (Fig. 1c). After depositing an egg, the hole was closed with a secretion. This secretion was visible as a very thin, transparent film layer exactly covering the hole. Adult feeding holes were similar, but were never sealed by a film. This character is useful for determining the presence of an egg without having to dissect the capitulum. In the field, females preferred to oviposit in mature capitula that have not yet bloomed (stage Bu-4 (Maddox 1981)) and rarely in those beginning to show florets (F-1). In the field, capitula containing an egg never had other feeding holes, and we observed no more than one egg in a capitulum. However, under confinement in our field sleeve cage experiment, we occasionally found two eggs in a capitulum, and we occasionally observed repeated feeding with up to three holes in a capitulum. In the cages, eggs were sometimes deposited among florets, but in the field they were only found in receptacle tissue (Fig. 1d). Eggs were common from mid-June to mid-July (12 June 2003–10 July 2003) in the field (Fig. 5). Eggs enclosed in 12.6 ± 2.6 (SD) d (range 9–17, $n = 8$) at room temperature ($\approx 22^\circ\text{C}$).

In the field survey, the first larvae were observed on 3 July 2003 (in Bingöl region, stage L1 and L2, $n = 11$; Fig. 5). In the field cages, the first larva (L1 stage) was seen on 5 July 2003, hatching from an egg. The larva started feeding on receptacle tissue and enlarged a chamber, eventually consuming ovaries, receptacle, and some other flower parts (Fig. 1e). Larvae fastened together the remaining flower parts and frass to form a pupal chamber inside the capitulum (Fig. 1f). Only one larva could complete development in a capitulum, even when more than one egg was deposited, such as sometimes occurred when females were confined in cages. Larvae were commonly seen from the beginning of July to the middle of September (15 September 2003) in the cages. However, a few larvae were seen up to October (9 October 2003) in the cages, but these larvae ($n = 3$) could not complete development, probably because it was too cold at this time of year (daily maximum 12°C and minimum $2\text{--}3^\circ\text{C}$). Larval devel-

opment time lasted ≈ 40 d, but was not directly studied. Larvae pupated inside the capitulum ≈ 3 d (range, 3–5) after they ceased feeding. The pupal stage lasted (16.6 ± 2.1 d, range 14–21, $n = 8$) at room temperature. Only one pupa occurred in a capitulum and was always positioned in a head-up position.

It was easy to distinguish from the outside which capitula contain a pupa or teneral adult because the capitulum dried as a mature capitulum (stage Bu-4 or F-1) and never extended the florets (flower stage F-2; Fig. 1h). We also reared three adult *Bangasternus orientalis* Capiomont and five adult *Larinus curtus* Hochhut from capitula, but capitula infested by these species lack the oviposition hole typical of *L. filiformis*. Adult *L. filiformis* waited inside capitula for about one week before emerging from the top of the capitulum. The first teneral adult *L. filiformis* inside a capitulum was seen on 22 July 2003 in Iğdır region (Fig. 5). Young adults were seen until the middle of September (16 September 2003) at the Iğdır site and until 13 October in the cages at Erzurum. Each newly emerged adult walked slowly and tried to find a hiding place. We never observed feeding or mating by the newly emerged adults of either *L. filiformis* or any other members of the genus *Larinus* that we observed. We released seven newly emerged adult *L. filiformis* outdoors and observed that they preferred to hide in soil under a brick, generally two to three individuals together (16 September 2003). We released 42 newly emerged adults from 11 to 16 September 2003 in a cage with various substrates (see Materials and Methods). The adults exhibited hiding and aggregation behavior under plant parts, a stone, or a brick. All individuals started to find a hibernation place between 11 and 16 September. ≈ 30 individuals aggregated in one place (Fig. 1i) were under a *Crambe orientalis* stem, a second group of $\approx 10\text{--}15$ individuals aggregated under the brick, partly entering the soil, and others were hidden in groups of 3–5 individuals under various objects and soil. *L. filiformis* has only one generation per year in northeastern Anatolia.

Dissection of Field-Collected Capitula

We dissected 695 *C. solstitialis* capitula that were periodically collected from natural habitats at three locations in eastern Turkey between 19 May and 16 September 2003 (Table 3). Adult feeding by *L. filiformis* on capitula was restricted to the premature (Bu-3; 61% of such capitula) and mature (Bu-4; 7%) developmental stages. The percentage of the premature capitula that were eaten increased steadily over time from 30 to 100%, suggesting that feeding on this stage arrested capitulum development, thus causing such capitula to accumulate as the season progressed, whereas undamaged capitula continued developing to other stages. Eggs were found only in mature capitula (Bu-4), with up to 42% of such capitula receiving eggs in mid-June. Larvae were found primarily in mature and preflowering capitula, and pupae and adults only occurred in preflowering capitula. Overall infestation rate of *C. solstitialis* capitula was 52%, based on the combined

Table 3. Proportion of *C. solstitialis* capitula containing different stages of *L. filiformis* collected at three field locations in eastern Turkey on different dates during 2003

Capitulum stage ^b	<i>L. filiformis</i> stage ^c	Location and sample date (mo/d) ^a											Total
		B	B	B	T	B	B	T	T	T	W	T, W	
		May 19	May 26	June 8	June 13	July 3	July 10	July 22	Aug. 1	Sept. 11	Sept. 12	Sept. 16	
0	Uninfested		75%										75%
0	Feeding		25%										25%
0 Total			24										24
2	Uninfested			70%	47%	27%	0%						39%
2	Feeding			30%	53%	73%	100%						61%
2 Total				30	38	30	22						120
3	Uninfested			80%	58%	65%	68%						68%
3	Feeding			20%	0%	8%	2%						7%
3	Egg			0%	42%	13%	2%						9%
3	Larva			0%	0%	15%	27%						16%
3 Total				20	12	40	44						116
4	Uninfested					80%	85%	0%	0%	0%	0%	0%	6%
4	Larva					20%	15%	17%	0%	2%	0%	1%	3%
4	Parasitoid					0%	0%	33%	51%	63%	51%	61%	52%
4	Pupa					0%	0%	33%	0%	2%	3%	1%	3%
4	Adult					0%	0%	17%	48%	34%	46%	36%	35%
4 Total						5	13	12	33	64	39	67	233
5	Uninfested							100%	100%	100%	100%	100%	100%
5 Total								13	11	17	57	104	202
Grand total			24	50	50	75	79	25	44	81	96	171	695
Total damaged by <i>L. filiformis</i> ^d			25%	26%	50%	49%	48%	48%	75%	79%	41%	39%	48%
Total infested by <i>L. filiformis</i> ^e			0%	8%	10%	20%	20%	48%	75%	79%	41%	39%	37%
<i>L. filiformis</i> parasitized ^f						0%	0%	33%	52%	63%	51%	61%	52%

On 19 and 26 May at Bingöl, *C. solstitialis* was still in the rosette stage, which lacks capitula, and adult feeding on leaves was observed. Damage reported includes both that by adults to capitula, or to rosettes when capitula were lacking, and damage by immatures to capitula. Infestation of capitula by immatures and parasitism of immatures are also reported.

^a B, Bingöl; T, Iğdır-Tuzluca; W, Iğdır-western slope Ağrı Mt.
^b Capitulum stages: 0, rosette; 2, premature (Bu-3) (Maddox 1981); 3, mature (Bu-4); 4, flowering (F-1 and two F-2); 5, postflowering (dry capitulum); total, no. of capitula examined.
^c Uninfested, no feeding hole or egg; feeding, adult feeding damage; egg, larva, pupa, adult, stage at time of dissection.
^d Including adult feeding of capitula or rosettes and presence of eggs, larvae, pupae, parasitoids, or adults inside capitula.
^e Including presence of eggs, larvae, pupae, parasitoids, or adults.
^f As a proportion of capitula containing larvae, pupae, parasitoids, or adults.

data from 22 July to 16 September (after the end of oviposition). The absence of infestation of “postflowering” capitula suggests that infestation prevents the capitulum from reaching this stage of development.

Natural Enemies

Signs of parasitization of *L. filiformis* larvae and pupae were first observed on 22 July. The highest parasitism rate observed was 63% of capitula that contained *L. filiformis* developed to at least the larval stage, which occurred on 1 August (Table 3). Over the full season, 52% of *L. filiformis*-infested capitula were parasitized, but omitting data before 22 July, when the first parasitism was observed, raised the estimate to 57%. Of the 122 parasitoids observed, 89% were associated with *L. filiformis* larvae and 11% with pupae. Parasitoids included: *Bracon urinator* (F.), *B. tshitscherini* Kok. (Braconidae), *Exeristes roborator* Fab. (Ichneumonidae), *Aprostocetus* sp. (Eulophidae), and unidentified species of Eurytomidae and Ormyridae. The most abundant parasitoid was *B. urinator*. The known hosts of *B. urinator* are all curculionids that mainly feed inside the capitulum of plants in the tribe Cardueae, but there is one record from the seed cap-

sule of an umbellifer, *Prangos uloptera* DC. (Table 4). *Exeristes roborator* attacks a wider variety of hosts, representing three orders, including both internal and external feeding larva, which occur on plants in eight families. *B. urinator* is not known to occur in North America; and although *Exeristes roborator* was released many times for biological control of *Ostrinia nubilalis* (Hubner) on maize and *Pectinophora gossypiella* (Saunders) on cotton from the 1920s to 1970s, it apparently has not persisted (Baker and Jones 1934, Baker and Bradley 1940, Legner and Medved 1979). The high rate of parasitism that we observed suggests that if *L. filiformis* was released in a new region with similar climate, lacking such parasitoids, the agent may become more abundant than in its native region. In North America, little parasitism of curculionids developing in capitula of *C. solstitialis* has been observed (Turner et al. 1990, Pitcairn et al. 2008), which is favorable for the potential effectiveness of *L. filiformis* on that continent.

No mortality of eggs was observed in the field. Some larvae that appeared to have died from pathogens were sent to Dr. M. Figen Dönmez for identification of possible entomopathogens. Two species of bacteria, *Pantoea agglomerans* and *Enterobacter cloacae*, were

Table 4. Known host species of parasitoids reared from *L. filiformis* in our study

Parasitoid/host species	Stage of host ^a	Host plant record ^b	References
<i>Bracon urinator</i> (Fabricius) Coleoptera, Curculionidae: <i>Larinus iaceae</i> (Fabricius)		<i>Cirsium palustre</i> , <i>C. oleraceum</i> , <i>C. arvense</i> , <i>C. lanceolatum</i> , <i>C. canum</i> , <i>Carduus nutans</i> , <i>Centaurea scabiosa</i>	Scherf (1964)
<i>Larinus sibiricus</i> Gyllenhal	L	<i>Xeranthemum annuum</i> L.	Gültekin and Korotyaev (2005)
<i>Lixus obesus</i> Petri	L, P	<i>Prangos uloptera</i> DC.	Gültekin (2005)
<i>Rhinocyllus conicus</i> (Frölich)		<i>Cirsium palustre</i> , <i>C. anglicum</i> , <i>C. arvense</i> , <i>C. lanceolatum</i> , <i>Carduus nutans</i> , <i>C. crispus</i> , <i>C. pycnocephalus</i> , <i>Centaurea nigra</i>	Scherf (1964)
<i>Exeristes roborator</i> (Fabricius) Coleoptera, Curculionidae: <i>Larinus brevis</i> (Herbst)		<i>Carlina acaulis</i> L.	Scherf (1964)
<i>Larinus capsulatus</i> Gültekin	P	<i>Echinops orientalis</i> Trautv.	Gültekin (2008)
<i>Larinus iaceae</i> (Fabricius)		<i>Cirsium palustre</i> , <i>C. oleraceum</i> , <i>C. arvense</i> , <i>C. lanceolatum</i> , <i>C. canum</i> , <i>Carduus nutans</i> , <i>Centaurea scabiosa</i>	Scherf (1964)
<i>Larinus latus</i> (Herbst)	L, P	<i>Onopordum bracteatum</i> Boiss. et Heldr.	Kasparyan and Gültekin (2002), Gültekin et al. (2003)
<i>Larinus onopordi</i> (Fabricius)	P	<i>Echinops</i> ? <i>pungens</i> Trautv.	Kasparyan and Gültekin (2002)
<i>Larinus</i> sp. pr. <i>remissus</i> Faust	P	<i>Centaurea</i> sp.	Kasparyan and Gültekin (2002)
<i>Lixus bardanae</i> (Fabricius)	P	<i>Rumex crispus</i> L.	Kasparyan and Gültekin (2002), Gültekin et al. (2004)
<i>Lixus cardui</i> Olivier			Kolarov (1997)
<i>Lixus obesus</i> Petri	L, P	<i>Prangos uloptera</i> DC.	Gültekin (2005)
<i>Rhinocyllus conicus</i> (Frölich)	L, P	<i>Cirsium uncrainicum</i> Bess., <i>Cirsium</i> sp.	Volovnic (1994), Kasparyan and Gültekin (2002)
Cynipidae, Hymenoptera <i>Diplolepis mayri</i> (Schlechtendal)		<i>Rosa</i> spp., <i>Anchusa leptophylla</i> Roem. and Schult.	Çoruh and Özbek (2008)
Lepidoptera, Lasiocampidae <i>Malacosoma neustria</i> L.		<i>Quercus</i> spp.	Çoruh and Özbek (2008)
<i>Malacosoma franconica</i> (Denis and Schiffermüller)		<i>Berberis vulgaris</i> L.	Çoruh and Özbek (2008)
Lepidoptera, Pyralidae <i>Ostrinia nubilalis</i> Hbn.			Kolarov (1997)
Lepidoptera, Tortricidae <i>Rhyacionia pinicolona</i> (Doubleday)		<i>Pinus sylvestris</i> L.	Çoruh and Özbek (2008)

^a L, larva; P, pupa.

^b The plant genera *Carduus*, *Carlina*, *Centaurea*, *Cirsium*, *Echinops*, *Onopordum*, and *Xeranthemum* are all in the tribe Cardueae of the family Asteraceae, which includes *C. solstitialis*. The remaining genera belong to other families: *Anchusa* (Boraginaceae), *Berberis* (Berberidaceae), *Pinus* (Pinaceae), *Prangos* (Apiaceae), *Quercus* (Fagaceae), *Rosa* (Rosaceae), and *Rumex* (Polygonaceae).

isolated from insects collected in the Bingöl region. Mortality from causes other than parasitoids at the larval and pupal stages was 1.7% and 1.3%, respectively. Therefore, total mortality of larvae and pupae was ≈60%. High parasitism rates such as we observed have not been reported in North America on other introduced insects feeding on capitula of *C. solstitialis*, including *Eustenopus villosus* (Boheman) (Curculionidae), which has a similar life history to *L. filiformis* (Piper 2001, Pitcairn et al. 2005). Parasitism of *E. villosus* in its native range in Greece was 30–46% (Fornasari and Sobhian 1993). Because *E. villosus* is one of the most effective agents established in California (Pitcairn et al. 2005), *L. filiformis* also could be a highly effective classical biological control agent if released in a region that lacks its parasitoids (Smith 2004a).

Adult Feeding Behavior Experiments

I. Adult Feeding on Host. *Larinus filiformis* adults fed on capitula of all three populations of *C. solstitialis*

tested. The attack rates (proportion of capitula damaged) were highest on the Çat (100%) and California (86%) plants and significantly less on Aşkale (61%) ($\chi^2 = 15.3$; df = 2; $P < 0.001$). The total number of capitula exposed were 84, 64, and 9 for Aşkale, California, and Çat, respectively. The low number of capitula available on Çat plants may have increased the attack rate relative to that on Aşkale and California plants. The relative proportion of capitula of different developmental stages exposed during the experiment did not differ among the three plant types ($\chi^2 = 14.8$; df = 10; $P > 0.05$). Overall, the proportions of capitula were 24.7% in bud stage (Bu-1 or Bu-2), 38.6% in premature stage (Bu-3), 19.0% in mature stage (Bu-4), 7.0% in preflowering stage (F-1), 10.1% in flowering stage (F-2), and 0.6% in postflowering stage (dry capitulum). Disregarding Çat plants, because they had few capitula, attack on California plants was higher than on Aşkale plants ($\chi^2 = 11.4$; df = 1; $P < 0.001$). In experiment II, the attack rate on California plants (95.2%) was also higher than on Aşkale plants (79.5%;

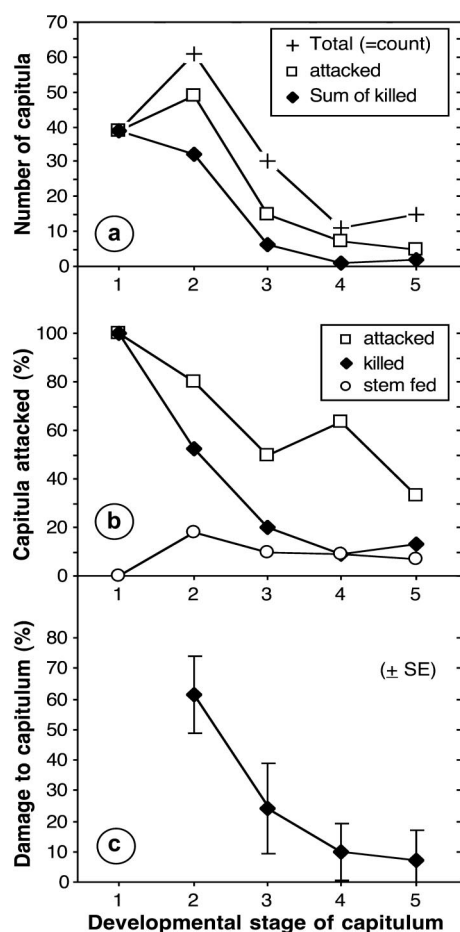


Fig. 6. Feeding preference of preovipositional adult *L. filiformis* on different developmental stages of capitula of *C. solstitialis*: (a) number of capitula available, attacked, and killed; (b) percentage of the capitula within each developmental stage that were attacked, killed, or fed on in the stem below the capitulum; and (c) percentage of tissue consumed inside capitula that were attacked. Capitulum stages: 1, bud (Bu-1 or Bu-2); 2, premature (Bu-3); 3, mature (Bu-4); 4, preflowering (F-1); 5, flowering (F-2); 6, postflowering (dry capitulum).

$\chi^2 = 7.8$; $df = 1$; $P < 0.01$; see below) but not different from Çat plants (90.0%; $\chi^2 = 0.5$; $df = 1$; $P > 0.1$). These results indicate that the California plants are at least as suitable as the Turkish plants for adult feeding.

Capitula that were available to the insects during this experiment were primarily in the bud and premature stages of development (Fig. 6a). Adults fed on a much higher proportion of capitula at the bud (100%) and premature (82%) stages than on the more mature capitula (17–54%), indicating a preference for younger capitula (Fig. 6b). Adult feeding killed 100% of buds, 56% of premature capitula, and progressively less of older capitula. Feeding on the stem below capitula occurred on only 0–18% of the plant tips. The proportion of tissue inside capitula that was consumed by adults decreased with increasing maturity of the

capitulum, probably reflecting the increasing size and decreasing palatability of capitula as they matured (Fig. 6c). Both sexes fed on capitula. In general, there was no feeding damage on leaves when capitula were present, otherwise adults can feed on rosette leaves, as they do in early spring in the field.

No eggs were observed to be oviposited during this experiment. It is probable that all the females were still in the preoviposition stage. The experiments ended on 26 June, whereas the eggs in the field were first observed on 12 June. The experiments were conducted in a climate chamber at 12:12 h photoperiod, which may have inhibited oviposition. Natural daylength at Erzurum in mid-June is ≈ 14.9 h. Because feeding rates were unknown, it is possible that there were not enough suitable capitula (buds and premature stages) to nourish the females sufficiently to produce eggs, or mature capitula (Bu-4 and F-1) suitable for oviposition. Confinement with males in the cages may have also interfered with oviposition.

II. Choice Experiment—Single Female-Male Pair.

The following nontarget test plants generally did not bolt or form capitula during this experiment: *Centaurea americana*, *Ce. calcitrapa*, *Ce. rothrockii*, *Cirsium brevistylum*, and *Ci. occidentale* variety *candidissimum*. Because the results of experiment I showed that *L. filiformis* adults usually feed on or below capitula, these plants were omitted from full analysis. However, some feeding holes were observed on the leaves and/or apical meristem of *Ce. americana*, *Ce. rothrockii*, and *Ci. brevistylum*, but no feeding was observed on *Ci. occidentale* variety *candidissimum*. Only one capitulum of *Ce. calcitrapa* was exposed, and it was not attacked. *Larinus filiformis* adults attacked and killed a high proportion of the *Ce. solstitialis* capitula (68–96%) (Fig. 7a). Adult feeding on capitula of nontarget plants occurred only on *Ce. cyanus* (19%) and *Ca. pycnocephalus* (22%). The amount of tissue consumed inside attacked capitula was highest in *Ce. cyanus* and *Ce. solstitialis* from Aşkale, intermediate in *Ce. solstitialis* from California and Çat, and lowest in *Ca. pycnocephalus* (Fig. 7b). There was no attack on 24 safflower or 78 lettuce capitula. In retrospect, the exposure period was too long (5 wk) for the number of plants (two target and up to three nontarget species in suitable developmental stage) to provide a realistic measure of the host specificity of this insect. The exposure rate was 0.12 *C. solstitialis* plants per female per day, 0.06 *C. solstitialis* plants per adult per day, and 0.16 total plants per female per day. This probably is why we observed some attack on *Ce. cyanus* and *Ca. pycnocephalus* in this experiment (representing physiological host range) but not on *Ce. cyanus* and other close relatives in the field survey (above; representing ecological host range) (Cullen 1990). In future studies, we recommend shortening the exposure period and/or increasing the number of plants available to achieve an exposure rate of more than one plant per female per day. Under such conditions, we would expect the attack of nontarget species would be much less (Marohasy 1998, Edwards 1999).

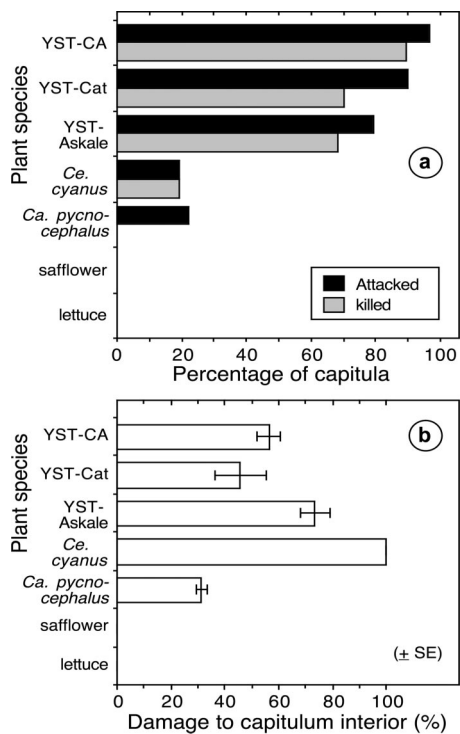


Fig. 7. Feeding by *L. filiformis* adults on capitula of different types of plants under choice conditions with at least two types of *C. solstitialis* present. (a) Percentage of exposed capitula that were attacked and destroyed. (b) Proportion of tissue consumed in capitula that had been attacked. (YST, *Ce. solstitialis*; accessions from: CA, California; Cat, Çat, Turkey; Askale, Aşkale, Turkey).

III. Choice Experiment—Multiple Females. We tested between 2 and 21 replicates of 11 nontarget plant species in the presence of the three types of *Ce. solstitialis* plants (Table 5; Fig. 1k). None of the non-

target test plants had begun to form capitula at the beginning of the experiment, so feeding damage was mainly limited to the leaves and apical meristems. The results for *Ce. cyanus* (bachelor's button), *Ca. pycnocephalus* (Italian thistle) and *Ca. tinctorius* (safflower) were most useful because these plants produced flowers during the experiment. Adult *L. filiformis* fed on leaves of all seven plants of *Ce. rothrockii*, and on some plants of *Ce. diffusa*, *Ce. stoebe* and *Ci. brevistylum* (Fig. 1m). Although *Ce. cyanus* and *Ca. pycnocephalus* were attacked in the single female-male pair choice experiment, there was no feeding on these plants in this experiment when more species of plants were present. These results suggest that the native North American plants, *Ce. rothrockii* and *Ci. brevistylum*, may be susceptible to adult feeding damage by *L. filiformis*. However, because the test plants had not bolted by the time of the experiment, it was not possible to evaluate oviposition preference. In this experiment the exposure rate was also too high (0.29 *C. solstitialis* plants per female per day, 0.13 *C. solstitialis* plants per adult per day, and 0.30 total plants per female per day), and probably overestimates the damage likely to occur in the field. Nevertheless, the results indicate no risk to safflower (*C. tinctorius*), which is one of the most important nontarget plants closely related to *C. solstitialis* (Smith 2007).

In conclusion, our results indicate, that under field conditions in eastern Turkey, *L. filiformis* is highly host specific, attacking only *C. solstitialis*. The laboratory choice experiments were less conclusive because plants were not at the optimal developmental stage; however, they suggest possible risk of adult feeding damage to leaves of some nontarget plants such as *Ce. rothrockii*, *Ce. cyanus* and *Ca. pycnocephalus*, under extremely high exposure rates. However, the primary damage caused by this insect is by larval and adult damage to the capitula and seeds, and the small amount of leaf feeding probably has little or no

Table 5. Multiple female choice adult feeding host plant preference test (11 females and 14 males one large cage (1 by 1 by 1 m) in a climate chamber for 18 d

Test plant	Developmental stage ^a	Number of plants tested	Plants with adult feeding damage (%) ^b	Location of feeding damage
<i>Carduus pycnocephalus</i>	B, P, F	4	0 [0, 60]	
<i>Carthamus tinctorius</i>	B, P, F	16	0 [0, 21]	
<i>Centaurea americana</i>	R	3	0 [0, 71]	
<i>Centaurea calcitrapa</i>	R	5	0 [0, 52]	
<i>Centaurea cyanus</i>	B, P, F	5	0 [0, 52]	
<i>Centaurea diffusa</i>	R	21	33 [15, 57]	Apical meristem
<i>Centaurea stoebe</i>	R	10	40 [12, 74]	Leaves
<i>Centaurea rothrockii</i>	R	7	100 [59, 100]	Leaves
<i>Centaurea solstitialis</i> (Aşkale)	B, P, F	20	70 [46, 88]	Apical meristem
<i>Centaurea solstitialis</i> (Çat)	B, P, F	6	100 [54, 100]	Apical meristem
<i>Centaurea solstitialis</i> (United States)	B, P, F	18	61 [36, 83]	Apical meristem
<i>Cirsium brevistylum</i>	R	7	29 [4, 71]	Apical meristem
<i>Cirsium occidentale</i> var. <i>candidissimum</i>	R	3	0 [0, 71]	
<i>Onopordum acanthium</i>	R	2	0 [0, 84]	

All plants were in rosette stage at the beginning of the experiment on 9 June 2003.
^a Developmental stages of capitula present on plants at end of exposure to adult insects: R, rosette (no capitula); B, young bud; P, preflowering; F, flowering stage.
^b Lower and upper 95% confidence intervals from binomial distribution in brackets.

impact. The high attack rates observed on *C. solstitialis* in the field, combined with the high level of damage to developing capitula by adult and larval feeding indicate that this agent should have high impact, similar to that observed for *Eustenopus villosus* (Pitcairn et al. 2005). Evidence to date indicates that this agent would probably be most effective at elevations >1,000 m and in the northern range of *C. solstitialis*, which may be beyond that preferred by the agents already established in the United States. Studies should be conducted in the United States to determine the abundance and impact of the previously released capitula insects at elevations >1,000 m and areas where snow occurs to determine if there is a need for *L. filiformis*. If there is a lack of effective agents at elevations of 1,000- to 1,400-m elevations, then further host plant specificity experiments should be conducted on *L. filiformis*, otherwise it is not certain that this insect would increase overall impact on *Ce. solstitialis* above that already achieved by *E. villosus* and *Chaetorellia succinea* (Costa) (Diptera: Tephritidae) (Pitcairn et al. 2004, 2005).

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